INTRODUCTION

The last twenty years have been marked by the immense development in the assisted reproductive techniques. Preimplantation genetic diagnosis was introduced to prevent the inheritance of sex linked diseases, the first successful pregnancy being achieved in 1990 (1).

PGD for aneuploidy screening (PGD-AS, PGS) aims to evaluate numerical chromosomal constitution of the cleavage stage embryo through removal of a blastomere/s and subsequent analysis by the use of fluorescence in-situ hybridization (FISH).

Apparently, approximately a third of all IVF produced embryos is chromosomally abnormal (2,3). In the poor prognosis IVF population which includes patients of advanced maternal age (AMA), recurrent implantation failure (RIF), recurrent miscarriage (RM) and testicular sperm extraction the incidence of chromosomal abnormalities rises to 70% (4).

The current morphological criteria for choosing the best embryo for transfer are often unable to allow selection of euploid embryos, i.e. morphologically best embryos are aneuploid in 25% of the cases (4). The rationale for introducing PGS has been that selection and transfer of euploid embryos would improve implantation, pregnancy rate and decrease miscarriage rate as well as multiple pregnancy rates. PGS is currently being done by an increasing number of infertility centers in the world and there is a need to assess its effectiveness (5).

Is it hype or hope?

A large number of comparative studies has investigated the use of PGS in patients of advanced maternal age (6-10), recurrent implantation failure (6, 7, 11, 12) and recurrent miscarriage (3, 13-15) and testicular sperm extraction (16, 17).

The results have showed initial optimism but a number of issues have to be addressed in order to interpret the findings and conclusions of different trials.

Certain methodological concerns such as sampling variability (wide array of sample sizes present) and clinical heterogeneity (different study populations, number of blastomeres assessed, number of probes used, methodology used, randomization procedures etc) are present in the studies assessing the use of PGS.

In accordance with the methodological concerns the Cochrane review (18) could only include two randomized prospective clinical trials (RCTs) Staessen et al (4) and Stevens et al (19). There is another small prospective randomized controlled trial by Werlin et al (20) but it does not provide sufficient data on the methodological quality.
The two randomized controlled trials included in the meta analysis (18), represent 428 patients with the majority of patients coming from the study of Staessen et al (n=389). PGS in the two studies was performed for advanced maternal age ≥ 37 in Staessen et al. and > 35 years in Stevens et al. There was no difference in the live birth rate in PGS vs. non PGS group, 11 vs. 15 % respectively (OR 0.65, 95% CI 0.36 to 1.19), ongoing pregnancy rate per woman 15% in the PGS group vs. 22 in control group, OR 0.42, 95% CI 0.12-1.51.

The trial by Werlin et al., randomized the three categories of patients, AMA, RM, RIF to PGS vs. non PGS group. Although this is the only study which randomized recurrent miscarriage patients, 11 vs. 8 controls, number of pregnancies 63.6% in the study vs. 37.5% in controls (p=0.07) it is difficult to interpret these results. The sample size was too small, the randomization procedure was not given and all patients received corticosteroids and low doses of aspirin. Regarding the patients with recurrent implantation failure (n=19, 11 study patients, 9 were controls) number of pregnancies was 20 % in the study vs. 0% in the control group.

Furthermore, results of the prospective cohort studies and retrospective studies showed that PGS improves the pregnancy rates but study design does not allow these results to be used for recommending PGS as a routine procedure.

A recent publication by Munne et al (21) analyzed 2279 PGD cycles which were carried out in patients older than 35 years from 100 infertility centers in USA. Majority of the centers had fewer than 30 cycles per centre, 89 of them, while number of cycles per centre ranged from 30 to 531 for the remaining 11 centers. In total, 1886 cycles ended in embryo transfer (82.2%), of which 608 resulted in pregnancy, but only 562 cycles with known pregnancy outcome were included. Results were compared with general IVF population, non donor fresh cycles, 7682 from 35-40 years and 1024 cycles > 40 years were used as a control group. The mean pregnancy loss for the PGS group (16.7%) was significantly lower than for general IVF group (21.5%, P< 0.001). When stratified for age, in the 35-40 group rate of pregnancy loss was 14.1 vs. 19.4 in the control group (P=0.03), and for patients over 40, it was reduced from 40.6 % in the control group to 22.2% (P<0.001). Although this trial has a large sample size as such, there was a large heterogeneity in the results of individual clinics, the pregnancy rate varying from 11 to 57%.

There have been no RCTs evaluating PGS in couples with NOA and OA although there is substantial evidence of an increase in aneuploidy and mosaicism of embryos derived from azoospermic men compared to fertile men (15-17). Surprisingly, Platteau et al showed that the aneuploidy frequency in embryos from NOA was 53% and in OA 60% despite young age of their female partners.

The results of all these studies confirm high rate of aneuploidies in these patient populations. A recent study of Baart et al (22), has shown that among young patients the rate of aneuploidies is 64% in embryos which were not selected for transfer.

Although extensive research has been conducted in this field there is a need for more well designed randomized prospective trials which will assess the value of PGS in well defined patient populations with delivery of a healthy child as the primary outcome.

Major limitation of PGS is mosaicism, estimate running as high as 50 % of all the cleavage embryos (23). Mosaicism is the result of presence of euploid and aneuploid cells or distinct aneuploidies on different blastomeres so that cells analyzed by PGS do not represent genomic content of the rest of the embryo. The mechanisms underlying this phenomenon are mitotic non disjunction and anaphase lagging. Coonen et al demonstrated that anaphase lagging accounts for 56% of the mosaicism in blastocysts (24).

Mosaicism often leads to misdiagnosis, up to 60%, (25) giving rise to false positive and false negative results. It has been argued that removal of only one blastomere is not representative of the embryo and two blastomeres need to be removed. Due to the fact that this removal is not carried in random order, when two blastomeres are analyzed, there is a 25% probability of removing both reciprocal daughter cells, resulting in the euploid status of previously mosaic embryo (22). There is also a chance of aggravating existing mosaicism by removal of normal blastomeres (26) and reducing number of healthy embryos for transfer.
It has to be reiterated that the current high incidence of mosaicism after PGS can be an overestimation since the embryos which have been analyzed in majority of the studies are discarded for transfer or cryopreservation. Staessen et al have shown that in patients of advanced maternal age the rate of mosaicism is 10.7% which is in agreement with the control group for recurrent miscarriage patients, by Pehlivan et al (11) of 10.8%. Although the populations studied are different, mosaicism rate was established in good quality embryos and as such may be more representative.

There are technical limitations of the procedure itself which have been acknowledged, namely signal overlapping and signal splitting (25).

Number of probes used varies among different groups, currently FISH is able to analyze up to 10 chromosomes, 1, 7, 13, 15, 16, 18, 21, 22, X, Y. Irrespective of the number of probes used not all chromosomes can be assessed by PGS at the moment. Comparative genomic hybridization (CGH) may overcome this, since it allows complete chromosomal status assessment, but there are still issues which prevent CGH from being routinely used such as long period of hybridization, necessity of embryo freezing prior to transfer, inability to distinguish diploid cells from haploid or tetraploid (27). The number of euploid embryos is lower than after FISH analysis, approximately 25% (28, 30). The first birth following CGH and a modified freezing protocol has been documented by Wilton et al in 2001(30).

In conclusion, PGS technique is still on the verge of hype and hope. Current clinical evidence shows no benefit in the pregnancy rates in poor prognosis patients but lack of well designed randomized controlled trials hinders definitive conclusions from being made.

Mosaicism of the cleavage embryos remains a great source of misdiagnosis, and can not be overcome by removing two instead of one blastomere.

The most important misinterpretation of the results is linked to the fact that the euploidy status of the blastomere doesn’t correspond to the euploidy status of the embryo due to mosaicism and probably due to the fact that the embryo is self correcting.

Finally, with the current technology available, including comparative genomic hybridization, screening of blastomeres will not lead to the evaluation of the entire embryo. It is foreseeable that if more randomized controlled trials will be available the final answer will confirm our interpretation.

REFERENCES


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